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A Physiological Comparison of Bivalve Mollusc Cerebro-visceral Connectives With and Without Neurohemoglobin. III. Oxygen Demand

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Abstract. Several bivalve mollusc species possess neurohemoglobin in their nervous systems whereas most species do not. The cerebro-visceral connectives of *Tellina alternata* and *Spisula solidissima* with neurohemoglobin and *Tagelus plebeius* and *Geukensia demissa* without neurohemoglobin exhibit similar electrical characteristics dictated mostly by axon size (0.3–0.4 μm mean axon diameter, Kraus *et al.*, 1988). Action potential conduction is sensitive to a depletion of both ambient and neurohemoglobin-bound oxygen. Connectives without neurohemoglobin and connectives with carbon monoxide neurohemoglobin ceased to conduct action potentials within 5–10 minutes after exposure to anoxic conditions. Connectives with neurohemoglobin conducted action potentials throughout the time course of neurohemoglobin deoxygenation, lasting 20–30 minutes.

Connectives without neurohemoglobin exhibited an approximate five-fold elevation in oxygen consumption rate during action potential conduction, as predicted by axon diameter. However, connectives with neurohemoglobin consumed only ⅓ this amount of oxygen during electrical activity. The mechanism for this increased efficiency in action potential conduction is unknown, but the rate of oxygen consumption nearly matches the rate of neurohemoglobin oxygen unloading *in situ*. An operational aerobic nervous system might enable animals to maintain neuromuscular activity during hypoxic or anoxic conditions.

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Introduction

Tissue hemoglobins supply oxygen to the surrounding tissue, and the ensuing aerobic metabolism produces energy which drives tissue function. In nervous tissue, the maintenance of ion gradients for action potential conduction requires energy to drive membrane-bound ionic pumps. Although the nervous systems of several organisms tolerant of anoxic conditions remain functional anaerobically (Mangum, 1980; Surkykke, 1983), total metabolic activity is sharply depressed (Ellington, 1981; Hochachka and Somero, 1984). In general, continued nervous activity is dependent on aerobic metabolism, as demonstrated in single axons (Maruhashi and Wright, 1967), compound nerves (Wright, 1946, 1947; Larrabee and Bronk, 1952), and brain cortex tissue slices (Bingmann *et al.*, 1984). For a review, see De Weer (1975).

Several species of bivalve molluscs possess substantial quantities of hemoglobin in their nervous systems, while most species do not. We have demonstrated that bivalve cerebro-visceral connectives with and without neurohemoglobin located in glial cells have similar electrical characteristics which are dictated by similar mean axon diameter (0.3–0.4 μm; Kraus *et al.*, 1988). In addition, both types of connectives possess comparable proportions of axon and glial cell volumes. However, axon bundles in cerebro-visceral connectives with neurohemoglobin are significantly smaller and contain fewer axons than in connectives without neurohemoglobin. This design provides more contact area and shorter mean distances between axons and glial cells. Thus, oxygen delivery from neurohemoglobin-containing glial cells to axons may be further enhanced (Kraus *et al.*, 1988).

Based on the oxygen affinity characteristics of the neurohemoglobin and the possibly low oxygen permeability of the perineural sheath, bivalve neurohemoglobin may

operate as an oxygen store for aerobic metabolism during anoxic conditions (Doeller and Kraus, 1988). Initial studies indicated that during anoxic conditions, the neurohemoglobin-containing cerebro-visceral connectives of *Tellina alternata* were able to conduct action potentials until neurohemoglobin deoxygenation was complete (Kraus and Colacino, 1986). To determine how effective the oxygen store would be, we needed to show that neurohemoglobin-containing connectives (1) depend on oxygen for action potential conduction and (2) consume oxygen at a rate that reasonably matches the neurohemoglobin oxygen unloading rate. In this investigation, we determined the oxygen demand of bivalve cerebro-visceral connectives with and without neurohemoglobin and their ability to conduct axon potentials in anoxic conditions.

Materials and Methods

Animals

Entire cerebro-visceral connectives were obtained from three species of bivalve molluscs with neurohemoglobin, *Tellina alternata, Spisula solidissima* and *Macrocalista nimbosa,* and two species without neurohemoglobin, *Tagelus plebeius* and *Geukensia demissa.* Animal collection (except of *M. nimbosa* collected from sandy mud flats at Long Beach, North Carolina), laboratory maintenance and cerebro-visceral connective dissection have been described previously (Kraus *et al.,* 1988).

Oxygen consumption rates

Basal and active oxygen consumption rates of entire cerebro-visceral connectives were measured with a flow-through respirometer similar in design to that used to measure the oxygen consumption rate of crab nerves (*cf.* Fig. 1, Baker and Connelly, 1966). To load the respirometer, a cerebro-visceral connective was inserted into a fluid-filled glass capillary. Bathing seawater (35 parts per thousand salinity) was drawn (Harvard syringe pump) from a reservoir into one end of the capillary and past the connective. The seawater PO_2, lowered by connective respiration, was detected by a polarographic oxygen sensor (Diamond Electrotec) at the other end of the capillary. The respirometer was housed within a temperature-controlled water jacket. All experiments were conducted at 15 ± 0.5°C for *S. solidissima* and 20 ± 0.5°C for the other species. The reservoir seawater was sterilized by filtration (0.45 μm, Millipore) or by near-boiling (approximately 90°C) in a microwave oven and equilibrated with air or air plus 3–5 mmHg PCO through an aeration port. This CO tension is sufficient to saturate the cerebro-visceral connective neurohemoglobin and remove its oxygen binding ability (Doeller and Kraus, 1988). The mass of the connective segment inside the capillary, responsible for the decline in oxygen tension, was computed as the product of the total mass of the connective and the ratio of the length of the connective in the capillary to its total length.

Capillaries of different bores were used to accommodate connectives of different diameters to minimize the fluid volume surrounding the connective. The flow rate through the capillary, which depended on connective and capillary diameters, was adjusted to reduce both the boundary layer at the oxygen sensor and the response time of the respirometer and at the same time to ensure an easily detectable drop in fluid oxygen tension. The sensor output was monitored continuously with a chart recorder or sampled at 5 Hz with an A/D-interfaced microcomputer (C.U. Electronics; OSI, Inc.). The microcomputer was programmed to calculate and record oxygen consumption rates graphically and numerically on a dot matrix printer (Epson) used as a chart recorder.

The initial oxygen consumption rate was often elevated, presumably due to the mechanical stimulation associated with loading the cerebro-visceral connective into the respirometer (see Fig. 1), but it usually stabilized within 20 min at a lower level denoted as the unstimulated or resting oxygen consumption rate. The connective was then stimulated at various pulse rates, 0.25–5.0 pulses per second, with supramaximal stimuli, approximately 5–10 V, at 1 ms duration. Stimulation was typically delivered for 5 min to allow seawater entering the top of the capillary at the onset of stimulation to pass over the entire connective while active. In this way, the declining oxygen tension of the seawater passing by the connective reached a relatively constant level before stimulation was terminated. The stable oxygen consumption rate at this time was denoted as the active rate. The connective was then allowed to resume its resting oxygen consumption rate before stimulation was repeated, which usually occurred within 0.5–1 h. Each connective was subjected to 4–8 stimulation bouts. Selected connectives were subsequently placed in a nerve-chamber gas-slide (*cf.* Fig. 1, Kraus *et al.,* 1988) to demonstrate continued electrical excitability after an experiment.

Action potential conduction

Individual anterior segments (25–30 mm long) of connectives with and without neurohemoglobin were suspended across a series of 12 platinum electrodes in the temperature-controlled nerve-chamber gas-slide. Stimulating electrodes were connected to a stimulator (Grass SD9) and recording electrodes were connected to a preamplifier (Narco) and microcomputer (Apple IIE) equipped with oscilloscope hardware and software (R.C.

Electronics). The slide was sealed with upper and lower glass lids and placed in the light path of a microspectrophotometer (Colacino and Kraus, 1984) in order to monitor the fractional saturation of neurohemoglobin (Doeller and Kraus, 1988). Gas ports connected the nerve-chamber to a gas delivery system. Connectives were thus exposed to different gas tensions while action potential conduction and, when present, neurohemoglobin fractional saturation were monitored simultaneously. The compound action potentials of cerebro-visceral connectives exhibit a predominant slow velocity spike associated with the vast majority of small diameter axons within each connective (see Fig. 6, Kraus *et al.*, 1988). The amplitude of this spike was used as the measure of action potential conduction.

During a stimulation bout, a connective segment was first exposed to flowing humidified air (100 ml/min). Action potential conduction was monitored and when neurohemoglobin was present, an absorption spectrum was recorded. After at least 15 minutes, the flowing gas was switched to humidified 99.999% N_2 and neurohemoglobin deoxygenation and action potential conduction were monitored. (Gas washout required only a few seconds because the volume of nerve-chamber plus input line was less than 1 ml.) When deoxygenation was complete and/or action potential conduction ceased, the connective was re-exposed to humidied air. After rapid neurohemoglobin reoxygenation and the return of electrical excitability, usually within 15 min, the experiment was repeated. During experiments with 3–5 mmHg PCO present, a carbon monoxide neurohemoglobin spectrum was recorded before switching to N_2.

Ouabain was used in several experiments to inhibit the Na-K pumps during action potential conduction. After electrical activity was demonstrated, a drop of filtered seawater with 1 m*M* ouabain was placed on a connective between the stimulating and recording electrodes. At the end of a 30 min equilibration period, the connective was stimulated at 1 pulse per second with 5–10 V, 1 ms duration, and compound action potential amplitude was recorded.

Results

Oxygen consumption rates

Resting and active oxygen consumption rates of bivalve cerebro-visceral connectives with and without neurohemoglobin are shown in Figure 1. The short delays seen between the start of stimulation and initial increase in the consumption rate and between the end of stimulation and initial decrease in the consumption rate may be attributable to the transit time of the slowly flowing seawater past the connective to the oxygen sensor. The

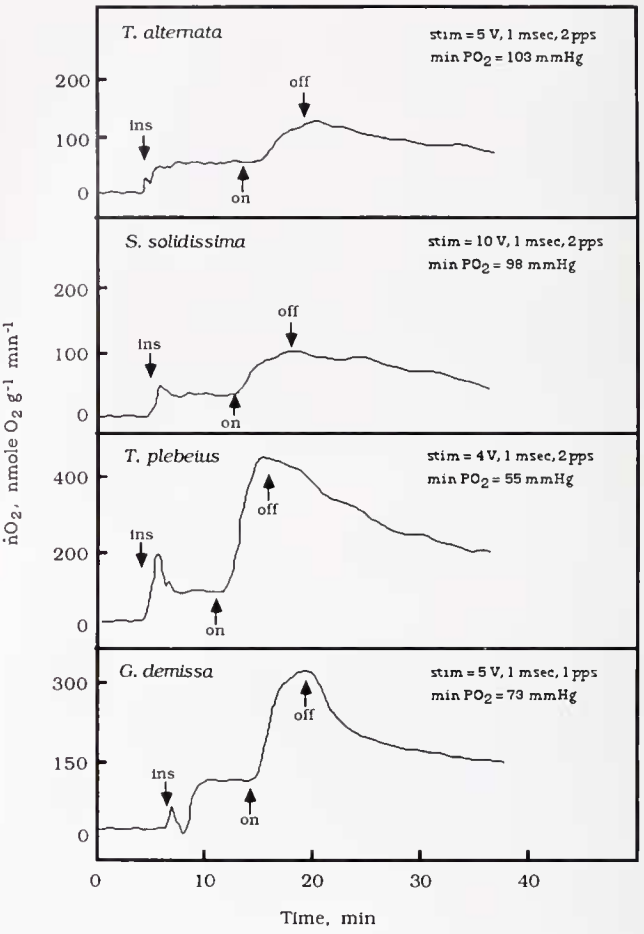

Figure 1. The resting and active oxygen consumption rates of bivalve cerebro-visceral connectives. The arrows refer to the times at which each connective is inserted into the respirometer (ins), the stimulation begins (on) and ends (off). The stimulation voltage, duration, and frequency and the minimum PO_2 are presented in the upper right hand corner of each trace.

differences in resting oxygen consumption rates were not significant, but connectives without neurohemoglobin appeared to consume oxygen at a slightly higher rate than connectives with neurohemoglobin ($0.1 < P < 0.25$, hierarchical ANOVA; Fig. 1, Table I). Inherent or spontaneous low-level electrical activity in the unstimulated connectives could contribute to the resting oxygen consumption rates.

Upon stimulation, connectives with and without neurohemoglobin all displayed an increase in oxygen consumption rate (Fig. 1, Table I). Because externally recorded compound action potentials displayed similar amplitudes in all preparations, the applied supramaximal stimulus was assumed to recruit as many axons as were excitable. Even so, the oxygen consumption rates of connectives without neurohemoglobin increased to a

Table I

Oxygen consumption rates of bivalve cerebro-visceral connectives

Pulses per second	Units	*Tellina alternata*	*Spisula solidissima*	*Macrocallista nimbosa*	*Tagelus plebeius*[a]	*Geukensia demissa*[a]
0	nmol O_2 g^{-1} min^{-1}	49.4 ± 16.1 (11)[b]	65.9 ± 19.6 (8)	48.8 (2)	83.0 ± 38.5 (15)	105.0 ± 46.8 (7)
2	nmol O_2 g^{-1} min^{-1}	101.4 ± 40.0 (5)	96.2 ± 14.3 (7)	126.0 (2)	505.2 ± 43.3 (5)	395.7 ± 67.1 (4)
2	nmol O_2 g^{-1} $pulse^{-1}$	0.84	0.80	1.05	4.21	3.30
2	nmol O_2 g^{-1} $pulse^{-1}$ above resting	0.43	0.25	0.64	3.52	2.42

[a] Species without neurohemoglobin.
[b] Numbers are given as average ± standard deviation (number of repetitions).

much greater extent than the rates of connectives with neurohemoglobin (Fig. 1, Table I). During experiments with neurohemoglobin-containing connectives, minimum PO_2 of the air-equilibrated seawater drawn through the capillary rarely fell below 100 mmHg, a magnitude greater PO_2 than required to fully saturate the neurohemoglobin *in situ* (Doeller and Kraus, 1988). In addition, the active oxygen consumption rates remained the same when the neurohemoglobin oxygen binding ability was eliminated with 3–5 mmHg PCO ($0.1 < P < 0.375$). The resting and active oxygen consumption rates of connectives without neurohemoglobin were not affected by this partial pressure of carbon monoxide ($0.1 < P < 0.375$).

The oxygen consumption rates of connectives with and without neurohemoglobin generally increased with increasing stimulus frequency (Fig. 2). Connectives without neurohemoglobin exhibited maximum oxygen consumption rates at a stimulus frequency of 2–3 pulses per second (Fig. 2). At higher stimulus rates, fewer axons may be conducting action potentials, as evidenced by a decrease in compound action potential amplitude, and this may lead to the observed decline in oxygen consumption rates. In contrast, connectives with neurohemoglobin exhibited nearly constant oxygen consumption rates from 0.5 to 5 pulses per second (Fig. 2). In an attempt to increase the consumption rates, stimulus strengths up to 50 V at 10 pulses per second were delivered, but action potential amplitude rapidly dropped as the connectives fatiqued and oxygen consumption rates actually declined.

Action potential conduction

Cerebro-visceral connectives with and without neurohemoglobin conducted compound action potentials at stimulus frequencies of 1–2 pulses per second for more than one hour under a flow of humidified room air, with less than a 10% change in amplitude or conduction velocity. At the onset of 99.999% N_2, compound action potential amplitude in connectives without neurohemoglobin rapidly declined and became indistinguishable from baseline in 4–8 minutes (Table II; Fig. 3). In contrast, action potential amplitude in connectives with neurohemoglobin remained elevated for about 20–30 min, or until the neurohemoglobin was nearly deoxygenated (Table II; companion neurohemoglobin deoxygenation traces lasted 18–26 minutes, see Fig. 3, Doeller and Kraus, 1988). When more than 90% of the neurohemoglobin oxygen store was effectively displaced by 3–5 mmHg PCO prior to N_2 exposure, action potential amplitude reached baseline in 5–10 minutes after N_2 exposure (Table II; Fig. 3). In air-equibrated conditions when ouabain was applied to directly inhibit Na-K pumps during stimulation, action potential amplitude of connec-

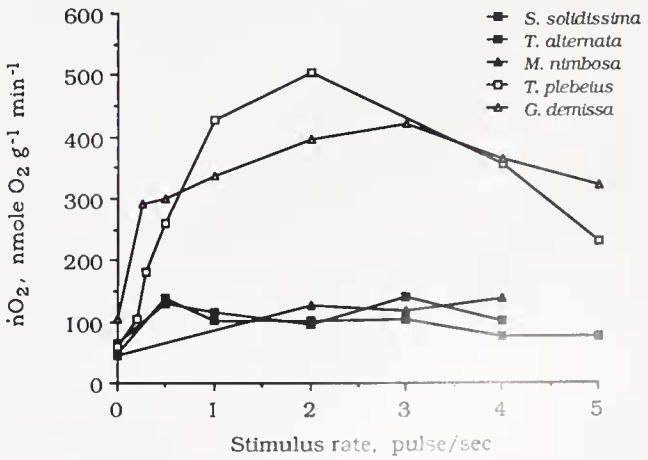

Figure 2. The effect of stimulus rate on the oxygen consumption rates of bivalve cerebro-visceral connectives. The closed symbols refer to connectives with neurohemoglobin, the open symbols refer to connectives without neurohemoglobin.

Table II

Time in minutes required for compound action potential amplitude of bivalve cerebro-visceral connectives to reach 50% of starting value after change in condition

Conditions	*Tellina alternata*	*Spisula solidissima*	*Tagelus plebeius*[a]	*Geukensia demissa*[a]
Air to N_2[b]	24.7 ± 6.0 (7)[c]	18.0 ± 3.8 (6)	3.9 ± 1.4 (4)	4.4 ± 1.9 (4)
Air + CO to N_2[b]	6.2 ± 1.3 (5)	6.3 ± 3.3 (4)	3.5 ± 0.7 (3)	5.5 ± 1.2 (3)
Air to air + 1 m*M* ouabain[d]	1.7 ± 0.8 (3)	2.5 ± 1.0 (3)	1.6 ± 1.3 (3)	2.3 ± 0.3 (3)

[a] Species without neurohemoglobin.
[b] Stimulus frequency 2 pulses per second.
[c] Numbers are given as average ± standard deviation (number of repetitions).
[d] After 30 min equilibration; stimulus frequency 1 pulse per second.

tives both with and without neurohemoglobin approached baseline in 5 minutes or less and electrical excitability was eliminated in the region of application of the drug (Table II).

Discussion

The basal oxygen consumption rate of any tissue represents the metabolic cost of cellular maintenance, and is characteristic of that tissue. In accordance, the mass specific resting oxygen consumption rates of nonmyelinated nerves do not vary greatly from one nerve type to another (Fig. 4; Ritchie, 1973). On the other hand, action potential conduction is a membrane phenomenon and the commensurate increase in oxygen consumption rate is a function of total axonal surface area (Ritchie, 1973). Compound nerves with small diameter axons contain more membrane surface area and possibly more energy-consuming Na-K pumps per gram than nerves with large diameter axons (Ritchie, 1973). Moreover, small diameter axons have a large surface area to volume ratio, and the dependency on active membrane-bound ionic pumps to maintain ion gradients may be high to compensate for the large fraction of ion content exchanged per action potential. Nonmyelinated bivalve cerebro-visceral connectives with and without neurohemoglobin, possessing 0.3–0.4 μm mean diameter axons, lose a calculated 0.05% of total cellular potassium per action potential (Kraus, 1986) whereas giant squid axons lose 0.000002% (Aidley, 1981). In experiments when Na-K pumps were inhibited by ouabain, connectives with and without neurohemoglobin lost electrical excitability in a short time, after conducting only a few hundred action potentials (Table II). Ouabain may interfere with the uptake of extracellular potassium by glial cells, as well as inhibit Na-K pumps (Johnston and Roots, 1972; Aidley, 1981). Thus, action potential conduction in bivalve connectives exhibits a strong dependancy on operational Na-K pumps which in turn rely on aerobic ATP production (Table II; see below). In fact, the magnitude of the increase in active oxygen consumption rate above resting is an inverse function of mean axon diameter, as shown in Figure 4. For example, the active oxygen consumption rate of giant squid axons is only a fraction greater than the resting rate (Connelly and Cranefield, 1953), but the active rate of small diameter garfish olfactory axons is many times greater than resting (Ritchie and Straub, 1979).

The resting and active oxygen consumption rates of bivalve cerebro-visceral connectives without neurohemoglobin are consistent with the trend displayed by other nonmyelinated nerves, based on axon diameter (lines c and e, Fig. 4). However, although the resting oxygen consumption rates of connectives with neurohemoglobin are similar to others, the active oxygen consumption rates are much lower than predicted by axon diameter (lines b, d, and f, Fig. 4) or total axonal surface area (Kraus *et al.*, 1988). Connectives with neurohemoglobin consume substantially less extra oxygen per impulse (2 pulses per second) than connectives without neurohemoglobin (Table I). Nonmyelinated garfish olfactory nerves, possessing 0.24 μm mean diameter axons and lacking neurohemoglobin, consume 2.46 nmol O_2 per g per impulse above resting (Ritchie and Straub, 1979), which is in close agreement with bivalve connectives without neurohemoglobin (Table I). The discordance in active oxygen consumption rates between connectives with and without neurohemoglobin was unexpected because both types of connectives exhibit very similar electrical and anatomical characteristics (Kraus *et al.*, 1988). Several possible explanations for this difference are proposed in the following discussion.

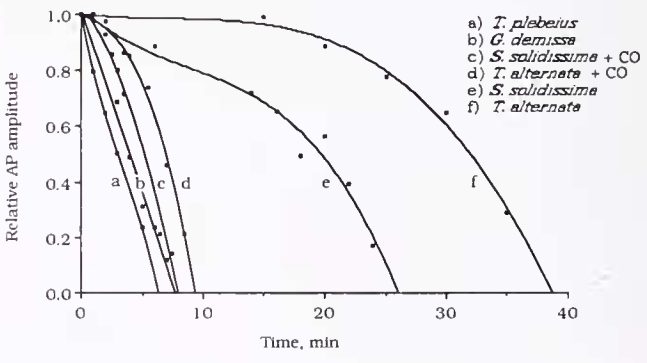

Figure 3. The relative action potential amplitude of bivalve cerebro-visceral connectives after the removal of oxygen at time 0, at 2 pulses per second. Lines a through d refer to connectives without neurohemoglobin or with an inactive neurohemoglobin, lines e and f refer to connectives with functional neurohemoglobin.

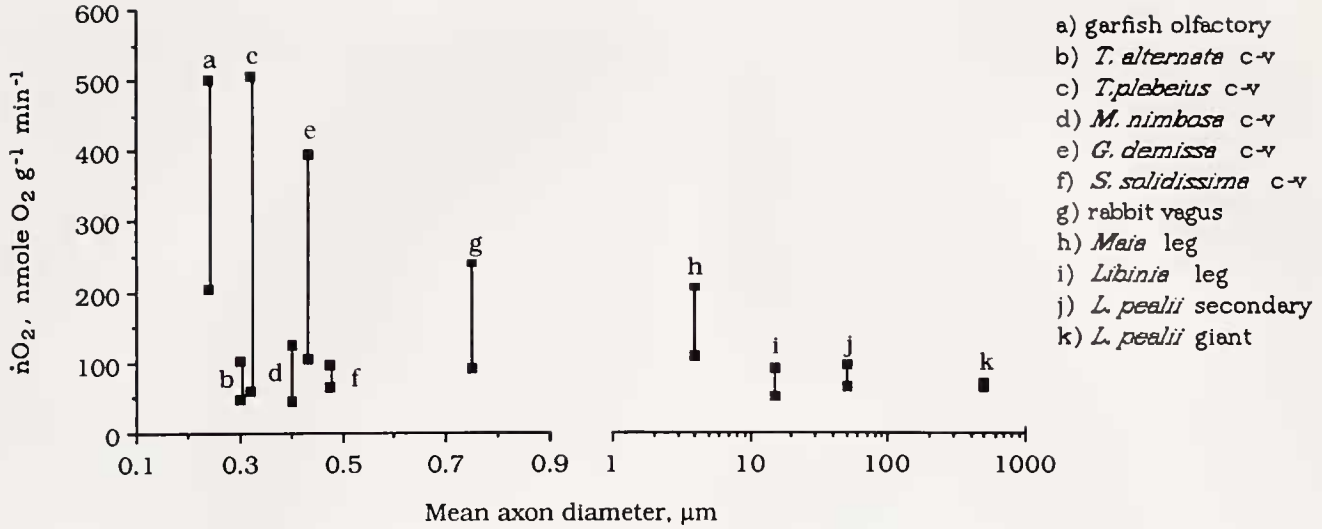

Figure 4. The effect of mean axon diameter on the resting and active oxygen consumption rates of nerves. Lower points refer to resting rates, upper points refer to active rates. Note the break in abscissa scale to accomodate a large range of axon diameters. Note also that lines c and e refer to bivalve cerebrovisceral connectives without neurohemoglobin, lines b, d, and f refer to connectives with neurohemoglobin. (a) rate, Ritchie and Straub (1979); diameter, Easton (1971); (b–f) rate, this study; diameter of b, c, e, f, Kraus *et al.* (1988) and d, estimated from other connectives; (g) rate, Ritchie (1967); diameter, Keynes and Ritchie (1965); (h) rate, Gerard (1932); diameter, estimated from Abbott *et al.* (1958); (i) rate, Baker and Connelly (1966); diameter, estimated from Baker (1965); (j) rate, Connelly (1952); diameter, estimated from Bullock (1965); (k) rate, Connelly and Cranefield (1953); diameter, Connelly (1952).

The maximum oxygen consumption rate of active nerves might be low if the energy requirements for action potential conduction were met by mechanisms that did not involve the consumption of oxygen from the surrounding medium. For example, if the neurohemoglobin-bound oxygen supply supported action potential conduction of connectives in the respirometer, then their measured oxygen consumption rates might have been artificially lowered. However, these neurohemoglobins begin to unload oxygen *in situ* at an ambient PO_2 of less than 10 mmHg, approximately the PO_2 where the rate of oxygen diffusion falls below the rate of oxygen consumption (Doeller and Kraus, 1988). Because the PO_2 of the bathing seawater in the respirometer rarely dropped below 100 mmHg during active respiration, the neurohemoglobin could not have been deoxygenated under these conditions. In experiments where neurohemoglobin fractional saturation was monitored during action potential conduction, the neurohemoglobin remained 100% oxygenated under air-equilibrated conditions, regardless of stimulus rate. In air-equilibrated seawater, resting and active oxygen consumption rates were not affected by carbon monoxide blockade of neurohemoglobin oxygen binding. We conclude that neurohemoglobin is not involved in supplying oxygen to support electrical activity at high ambient oxygen tensions.

Action potential conduction could also be less oxygen-dependent if the neurohemoglobin-containing connectives derived cellular energy from an anaerobic metabolic source. Both the sea anemone and the lugworm (which do not possess neurohemoglobin) can endure complete anoxia for days and still conduct action potentials or display complex sensory reflexes upon intermittent stimulation (Mangum, 1980; Surlykke, 1983). However, continuous action potential conduction by bivalve connectives with and without neurohemoglobin is quite sensitive to a depletion of both ambient and neurohemoglobin-bound oxygen. Under anoxic conditions, both the change in action potential amplitude of neurohemoglobin-containing connectives and neurohemoglobin deoxygenation follow the same time course (Kraus and Colacino, 1986; Doeller and Kraus, 1988). Connectives without neurohemoglobin and connectives with carbon monoxide neurohemoglobin both cease to conduct action potentials within 5–10 minutes after exposure to anoxic conditions (Fig. 3). Therefore, anaerobic metabolism cannot support action potential conduction at 1–2 pulses per second during anoxic conditions. Instead, the aerobic energy requirements of action potential conduction may be fundamentally different in the two types of connectives. Connectives with neurohemoglobin may consume less oxygen because they hydrolyze less ATP, assuming oxidative phosphorylation is equally efficient in both types of connectives.

Because the principle consumers of ATP in nervous tissue are the membrane-bound ionic pumps, less ATP

would be hydrolyzed if fewer ions were pumped after each action potential. This would occur if fewer ions crossed the membrane during each action potential. There are several mechanisms which might lead to a reduction in the quantity of ions exchanged per impulse, each of which requires investigation. First, the total concentration of participating ions within the connective could be reduced. Cerebro-visceral connectives of the osmoconforming mussel *Mytilis edulis*, acclimated to 25% seawater, showed an approximately 50% reduction in internal sodium and potassium concentrations (Willmer, 1978a), but resting and action potential amplitudes were identical to those from animals acclimated to 100% seawater (Willmer, 1978b). Reduced ionic concentrations may be maintained in part by solute substitution to maintain osmotic balance, a diffusion barrier, or accessory ionic regulation by glial cells. Solute substitution may involve amino acids or other solutes, as occurs in the osmoconformer *Elysia chlorotica* (Pierce *et al.*, 1983; Parker and Pierce, 1985). The densely laminated perineural sheath of neurohemoglobin-containing connectives (Kraus *et al.*, 1988) may resist the diffusion of ions in a manner similar to the perineural sheath of cerebrovisceral connectives in two freshwater mussels, which restricts the passage of sodium ions (Twarog and Hidaka, 1972). Because a diffusion barrier alone is not sufficient to maintain low internal ion concentrations, supplementary ion pumping, perhaps at the peripheral glial cell layer, may be necessary. To support axon function during action potential conduction, glial cells may regulate the ionic constituents of the intercellular fluid by limiting the rise of potassium (Somjen, 1975; Orkand, 1982) or replenishing depleted sodium (Sattelle and Howes, 1975). Regulation of this type may be essential in closely packed axon bundles where intracellular volume is considerably greater than intercellular volume (see Fig. 3, Kraus *et al.*, 1988; intercellular volume of cerebro-visceral connectives of *Mytilis edilus* is only 12–20% of total volume, Willmer, 1978a). Ionic regulation by the neurohemoglobin-containing glial cells may be enhanced by the greater surface area contact between glial cells and axons (Kraus *et al.*, 1988). Glial cell function may be an important factor in the reduction in active oxygen consumption rates.

Second, fewer ions may be involved in action potential conduction if the axon membranes possessed a lower ion channel density. Willmer (1978c), measuring tetrodotoxin sensitivity, showed that connectives from *Mytilus edulis* may undergo a seven-fold reduction in sodium channel density upon acclimation to 25% salinity. Hypoxia-tolerant tissues may reduce the density of functional ion channels to compensate for less ion pumping during hypoxic or anoxic conditions (Hochachka, 1986). The increased membrane resistance resulting from a lower ion channel density might not effect the cable properties of the axons if the longitudinal resistances of the extracellular fluid and axoplasm were increased as a result of lower ion concentrations. Therefore, action potential properties would not be affected. As has been noted, the compound action potential properties of connectives with and without neurohemoglobin are not significantly different (Kraus *et al.*, 1988).

The lowered maximum oxygen consumption rates enable connectives with neurohemoglobin to effectively use the oxygen stored on the neurohemoglobin. The maximum oxygen consumption rates of connectives from both *T. alternata* and *S. solidissima* are well-matched to the linear oxygen unloading rates of each neurohemoglobin: compare 101 ± 40 to 146 ± 38 nmole $O_2\ g^{-1}\ min^{-1}$ for the oxygen consumption rate and neurohemoglobin unloading rate of *T. alternata*, and 96 ± 14 to 100 ± 24 nmole $O_2\ g^{-1}\ min^{-1}$ for the oxygen consumption rate and neurohemoglobin unloading rate of *S. solidissima* (Doeller and Kraus, 1988). Higher consumption rates would deplete the neurohemoglobin oxygen store more rapidly under anoxic conditions and lower rates would allow some oxygen to diffuse out of the connective.

The ecophysiological significance of a nervous system that can function under anoxic conditions for an extended period of time may become apparent when one considers the lifestyles of the bivalves studied here. [The following descriptions are from Stanley (1970) and personal observations in the field and laboratory.] *Tellina alternata* is a smooth shelled, laterally compressed bivalve with rapid burrowing capabilities. It typically resides 10–20 cm below the sediment surface in sandy mud near the mean low tide mark of low energy beaches where much of the organic debris carried by swash currents tends to settle out. The sandy mud of its habitat is black to grey and smells strongly of sulfide, which is characteristic of anoxic sediment. Other species commonly found in this habitat are *Chaetopterus variopedatus, Notomastus lobatus,* and *Divariacella quadrisulcata.*

As a deposit feeder, *T. alternata* siphons organic particles from the sediment surface. During feeding, the animal has a ready supply of oxygenated water as it extends its long incurrent siphon to the sediment surface. However, once the sediment around its siphon is depleted of deposits and the animal retracts its siphon to burrow laterally to a new location, its source of oxygen is removed. Burrowing requires much foot and valve movement coordinated by an operational nervous system. Under anoxic conditions, *T. alternata* maintains a high metabolic rate (Kraus, 1986), most likely by using the anaerobic metabolic pathways found in many bivalve molluscs (de Zwaan, 1983). Therefore, an aerobic nervous system which is able to operate for 20 minutes or more under

anoxic conditions by using a neurohemoglobin oxygen store would allow *T. alternata* to burrow laterally for a distance of at least 1 m, at the observed rate of 5 cm/min, certainly far enough to locate fresh surface deposits.

Spisula solidissima is a large laterally compressed active clam. Although *S. solidissima* is a filter feeder with relatively short siphons, it does not inhabit a permanent burrow and often burrows rapidly through the sediment below the surface. Under these hypoxic conditions, its neurohemoglobin-containing nervous system could continue to operate to ensure the eventual return to a source of oxygen. *Macrocallista nimbosa* exhibits a morphology and lifestyle similar to *S. solidissima* and its neurohemoglobin probably operates in a similar fashion. Neither *S. solidissima* nor *M. nimbosa* burrow as deeply as *T. alternata* and their neurohemoglobins are not as concentrated.

Tagelus plebeius and *Geukensia demissa* are less active bivalves than *T. alternata* and *S. solidissima*. *T. plebeius* is an elongated cylindrical filter-feeding bivalve that burrows slowly. It is commonly found in sandy intertidal mud flats in communities with *Mercenaria mercenaria* and *Saccoglossus kowalewskii*. *T. plebeius* occupies a permanent cylindrical burrow which extends vertically to 50 cm or more below the sediment surface. At high tide, *T. plebeius* occupies the upper portion of the burrow and extends its siphons through two small openings to filter feed from the abundant food source in the overlying water column. As the tide ebbs, the animal withdraws its siphons to avoid predation and slowly moves down in the burrow to follow the water table. Under anoxic conditions, *T. plebeius* exhibits very low rates of heat production and oxygen consumption until it is reexposed to aerated conditions (Kraus, 1986). *Geukensia demissa,* another sedentary bivalve, is found securely attached by byssal threads to stationary debris near the sediment surface in backwater regions. During air-exposure, animals extract oxygen from the air, but deep tissues remain poorly oxygenated (Booth and Mangum, 1978).

In summary, the cerebro-visceral connectives of *T. alternata* and *S. solidissima* with neurohemoglobin, and *T. plebeius* and *G. demissa* without neurohemoglobin exhibit electrophysiological and ultrastructural characteristics which are similar to each other and to other nerves with small diameter axons (Kraus *et al.*, 1988). However, the connectives with neurohemoglobin consume much less oxygen during action potential conduction than connectives and other nerves without neurohemoglobin. This increased efficiency enables the neurohemoglobin-containing connectives to effectively use the neurohemoglobin oxygen store, which in turn may enable the animals to use continued neuromuscular activity during hypoxic and anoxic conditions.

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